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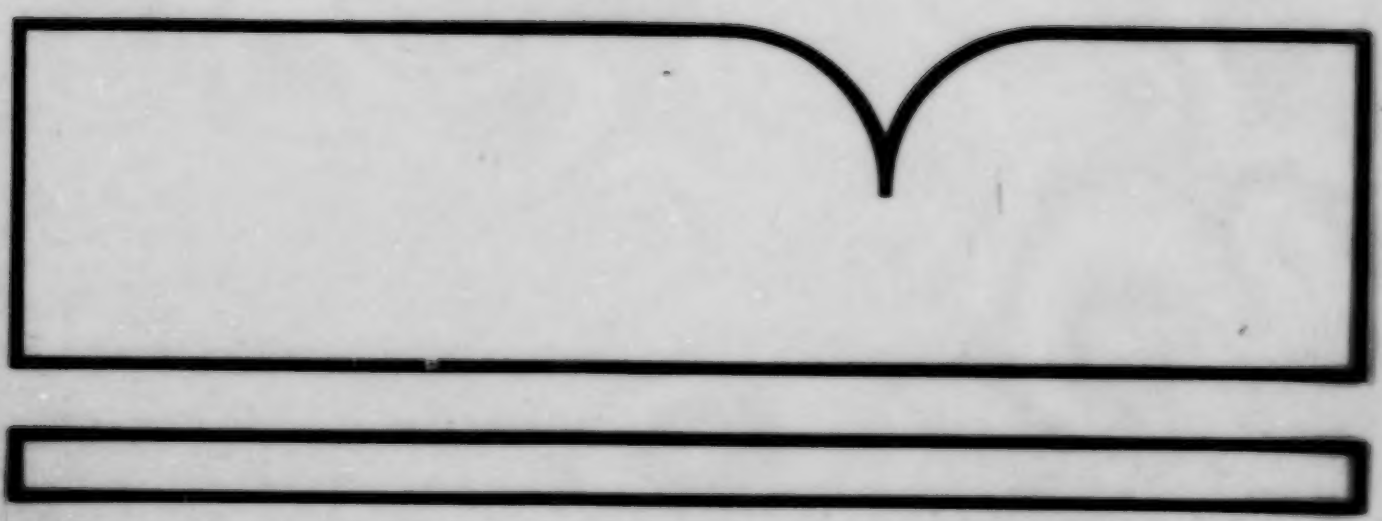

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16. ABSTRACT

By a previously established test procedure that uses 4.5 μM phosphate in the medium, the marine red alga *Champia parvula* (C. Agardh) Harvey was shown to be more sensitive to arsenite [AsIII] than to arsenate [AsV]. Mature cystocarps (evidence of sexual reproduction) were formed when plants were exposed to 65 μg AsIII/L, but not when exposed to 95 μg AsIII/L. Three hundred micrograms AsIII/L was sufficient to kill *C. parvula*. Using the standardized test, plants grew just as well in 10,000 μg AsV/L as they did in the controls; however, sexual reproduction did not occur at this concentration. Phosphate concentration up to 9.1 μM had little or no effect on AsIII toxicity. Arsenate toxicity, however, was inversely proportional to the amount of phosphate in the medium. In the absence of added phosphate, As(V) toxicity was similar to that of AsIII, except plants were still alive at 1,076 μg AsV/L (although they did not grow).

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TOXICITY OF ARSENITE AND ARSENATE TO THE MARINE MACROALGA *CHAMPIA PARVULA* (RHODOPHYTA)

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Abstract — By a previously established test procedure that uses 4.5 μM phosphate in the medium, the marine red alga *Champia parvula* (C. Agardh) Harvey was shown to be more sensitive to arsenite [As(III)] than to arsenate [As(V)]. Mature cystocarps (evidence of sexual reproduction) were formed when plants were exposed to 65 μg As(III)/L, but not when exposed to 95 μg As(III)/L. Three hundred micrograms As(III)/L was sufficient to kill *C. parvula*. Using the standardized test, plants grew just as well in 10,000 μg As(V)/L as they did in the controls; however, sexual reproduction did not occur at this concentration. Phosphate concentration up to 9.1 μM had little or no effect on As(III) toxicity. Arsenate toxicity, however, was inversely proportional to the amount of phosphate in the medium. In the absence of added phosphate, As(V) toxicity was similar to that of As(III), except plants were still alive at 1,076 μg As(V)/L (although they did not grow).

Keywords — Aquatic toxicology Arsenic Phosphate Macroalgae *Champia*

INTRODUCTION

Little is known of the sensitivity of aquatic plants to arsenic. In freshwater ponds, 2310 μg/L arsenite [As(III)] is sufficient to eliminate all of the dominant green algae and most of the angiosperm *Potamogeton* sp. [1]. Arsenate [As(V)] inhibits the growth of the freshwater green alga *Chlamydomonas* at 75 μg/L [2]. Growth of the marine planktonic algae *Tetraselmis chui* and *Hymenomonas carterae* is not affected by either As(III) or As(V) at concentrations as high as 1,000 μg/L, and they can survive in concentrations of As(V) up to 1,000 mg/L [3]. However, As(V) inhibits carbon uptake and growth in the marine diatom *Skeletonema costatum* at 5 and 12.5 μg/L, respectively, and As(III) had the same effects at similar concentrations [4]. The only work to date on the toxicity to marine seaweeds shows that the development of sporelings of the red alga *Plumaria elegans* is inhibited after 18 h of immersion in 1,000 μg As(III)/L [5].

Arsenic and phosphorus are similar in their chemical characteristics, and phosphate has been shown to influence the toxicity of As(III) and As(V) [4,6–9], as well as the level of arsenic in algal tissues [10]. In the

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present study, tests were performed to determine the relative sensitivity of *Champia parvula* to As(III) and As(V), and the influence of phosphate on their toxicities. *C. parvula* is sensitive to heavy metals (Ag, Cd, Cu and Pb), and is comparable in this sensitivity to the most sensitive marine animals [11].

MATERIALS AND METHODS

Toxicity tests followed standardized procedures previously established for the red alga *C. parvula* [11], except that EDTA was omitted from the medium and vitamins were added. In addition, only male and female gametophytes were used in these toxicity tests. Earlier results with heavy metals (Ag, Cd, Cu and Pb) and cyanide showed sexual reproduction of *C. parvula* to be the most sensitive part of the life cycle [11]. Sexual reproduction is also more sensitive than vegetative growth in the brown algae *Fucus edentatus* and *Laminaria saccharina* when exposed to petroleum products [12].

Unialgal stock cultures were maintained in 500-ml screw-capped Ehrlenmeyer flasks containing 400 ml of a modified medium "f" [13]. The medium consisted of 9.35 mg $NaNO_3$, 0.62 mg $NaH_2PO_4 \cdot H_2O$, 2.6 μg Fe (as Cl^-)[1] and "f/16" levels of vitamins (0.06 μg B_{12}, 0.06 μg biotin and 12.5 μg thiamine · HCl) per liter of filtered seawater (15 μm charcoal filter and 0.3 μm Balston filter). No silicate or trace metals were added to the cultures. Cultures were maintained on a rotary shaker at 100 rpm and were illuminated from above at a light intensity of 75 $\mu E\ m^{-2}\ s^{-1}$ of cool white fluorescent light on a 16 h light:8 h dark cycle. The temperature was 20 to 22°C. Media were changed once a week.

Toxicity tests lasted 14 d, and were run to examine (a) the relative sensitivity of *C. parvula* exposed to As(III) and As(V) under standard conditions (i.e., 4.5 μM phosphate), (b) the effect of phosphate concentration on the toxicity of As(III) and As(V) and (c) the effect of As(V) with no phosphate added. Test flasks and media volumes, as well as all other conditions, were the same as those used for the maintenance of stock cultures. Stock plants were rinsed in sterile seawater to remove traces of old media, and 2- to 3-mm branch tips (approximately 0.025 mg dry weight) were cut to serve as inocula. Five tips were placed into each test flask. In addition, one male branch (approximately 1 cm long), visibly producing spermatia, was added to each flask. When media were changed (on days 7 and 11) the male branches were clipped back to 1 cm to minimize competition with females for nutrients.

Range-finding and definitive experiments were performed for both As(III) and As(V). Range-finding experiments covered a broad range of concentrations (approximately 10 to 10,000 μg/L). Five to seven arsenic concentrations plus a control were used in each definitive run. All the definitive experiments were either performed in duplicate or repeated at least once. Reagent-grade arsenic (as sodium arsenite or sodium arsenate) was added directly to the culture flasks with digital micropipettes from a 10,000 mg/L stock solution, and was replenished when the media were changed.

The effect of phosphate on the toxicities of As(III) as As(V) was measured with concentrations of added phosphate ranging from 0 to 9.1 μM. For As(III), background phosphate was removed from natural seawater by precipitation with ferric chloride (100 mg/L) [14] at pH 9.5. The resulting floc was allowed to settle, the water decanted and filtered through a 0.3 μm Balston filter (pH adjusted to 7.8 with HCl) and autoclaved. Phosphate-free water was used to ensure that the AS(III) treatments with no added phosphate contained no background phosphate. Initial runs showed no effect of phosphate on As(III) toxicity; therefore, we wanted to eliminate the possibility of background phosphate being sufficient to inhibit As(III) toxicity. Plants grown in the iron-precipitated medium with

[1] A 1-mg/ml stock solution was prepared by dissolving 1 g iron powder in 10 ml concentrated HCl with low heat, and diluting to 1 liter with deionized water.

low added phosphate had a white precipitate (presumably $CaCO_3$) on their surfaces; therefore, these data are reported as ash-free dry weights. Plants were ashed overnight at 600°C on pre-ashed and preweighed glass-fiber filters. The effect of phosphate on As(V) toxicity was determined using seawater that was not precipitated with iron.

Arsenite was measured as total arsenic by heated graphite atomization (HGA) using a Perkin-Elmer Model HGA 500 coupled with a Perkin-Elmer Model 5000 atomic absorption spectrophotometer. Prior to analysis, 100 μl of concentrated HNO_3 (ultra-pure grade) was added to 1 ml of each of the test samples. Standards were prepared in control seawater to eliminate salinity differences between standards and test samples. The detection limit for arsenic with this technique is approximately 5 μg/L.

Arsenate, in a 10-ml sample, was measured by the colorimetric method of Johnson [15]. Concentrations of 3,600 μg/L and above were diluted 1:10 with deionized water.

At the end of each test, the plants were rinsed with deionized water, oven-dried at 80°C for 3 to 4 d and weighed ($\pm$0.01 mg). Differences in the amount of vegetative growth of females (final dry weight) and presence or absence of cystocarps (evidence of sexual reproduction) were used to assess toxicity. When mature cystocarps were not evident the presence or absence of immature cystocarps was noted. Immature cystocarps are evidence of sexual fusion, but no spores are present. Statistical significance for differences in vegetative growth was calculated using analysis of variance followed by Tukey-Kramer's multiple range test, $\alpha = 0.05$ [16].

RESULTS AND DISCUSSION

Using the previously established test procedures, the sensitivity of *C. parvula* to arsenic varied greatly, depending on the oxidation state of the arsenic. *C. parvula* is relatively sensitive to the presence of As(III) in seawater. Growth was significantly inhibited at 145 μg As(III)/L, and sexual reproduction ceased at concentrations between 60 and 95 μg/L (Fig. 1, top). All individuals died at concentrations of 300 μg/L and above. Sensitivity of *C. parvula* to As(V)

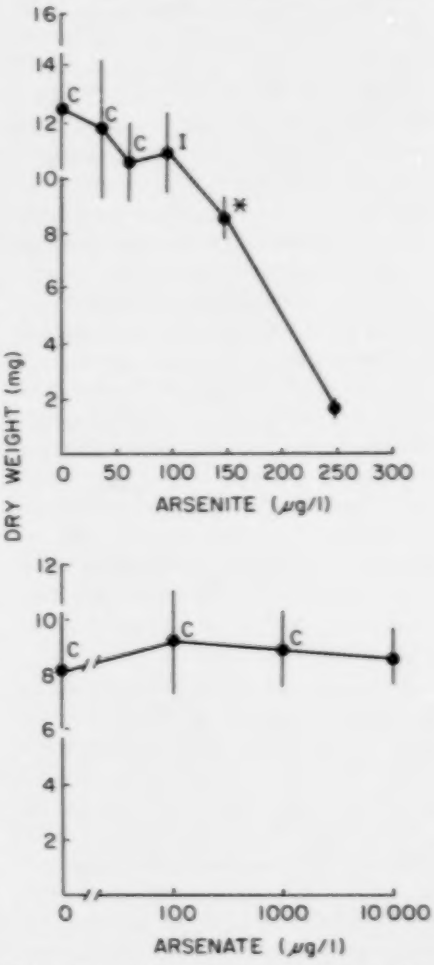

Fig. 1. Growth (n = 10) and sexual reproduction (C, cystocarps present; I, only immature cystocarps present) of *Champia parvula* in response to As(III) (arsenite; top) and As(V) (arsenate; bottom). Asterisk denotes the first treatment in which growth was statistically different from that in the control. Concentrations of As(III) in the water are means based on samples taken on day 0, before the medium was changed on days 7 and 11 and at the end of the experiment (day 14). As(V) concentrations were not measured for this run.

was much less than that to As(III) under the standard conditions (Fig. 1, bottom). An As(V) concentration of 10,000 μg/L did not significantly affect growth; however, sexual reproduction was eliminated at that concentration.

Control seawater flasks contained no detectable As(III) or As(V). The concentrations of total dissolved arsenic in As(III) treatments decreased only 2 to 8% over a 7-d period (the maximum amount of time between two consecutive medium changes), although some As(III) was certainly oxidized to As(V). In preliminary experiments, a delay of 24 h in adding plant material after the addition of As(III) significantly reduced the toxicity of As(III), presumably due to its oxidation to As(V) [As(V) concentrations were not measured in As(III) experiments]. Arsenate concentrations in As(V) experiments were only measured at the beginning of experiments, and occasionally after media were replaced and fresh As(V) was added. Therefore, loss of As(V), either by uptake or reduction to As(III) was not determined. If significant amounts of As(V) were reduced to As(III), however, there should have been a toxic response by the alga, which did not occur.

Phosphate had no effect on the response of vegetative growth to As(III) over the range of phosphate concentrations tested (Fig. 2). Growth at concentrations of As(III) of 113 μg/L and less was statistically the same as that for the control (no As(III) added) at all phosphate concentrations tested. At 212 μg/L, growth was significantly less than in the controls, and the inhibitory effect was independent of phosphate concentration. Plants in all phosphate treatments died at an As(III) concentration of 313 μg/L.

Arsenate toxicity was inhibited in the presence of phosphate (Fig. 3) without using phosphate-free water. When no phosphate was added to the medium, plants died in all treatments except the control (no As(V) added). At 4.5 μM added phosphate (the concentration used in the standardized procedure), growth in all As(V) treatments statistically was equal to that in the control.

Sexual reproduction in the phosphate experiments followed the same general pattern as vegetative growth with respect to As(III) and As(V) toxicities (Fig.4). At 54 μg As(III)/L there was a slight phosphate effect: only immature cystocarps were present at the two lowest phosphate concentrations and the higher phosphate concentrations allowed the formation of mature cystocarps. However, the inhibitory action of phosphate on As(V) toxicity was much more pronounced. Only at the highest As(V) concentration tested was phosphate not able to restore sexual reproduction.

The response of vegetative growth to As(V) was greatly increased in the absence of added phosphate in the medium (Fig. 5). Growth was significantly less than that in the control at the lowest As(V) concentration tested, 56 μg/L. This was less than the concentration of As(III) necessary to cause a significant decrease (Fig. 1, top). Survival, however, was better in As(V) than As(III) treatments. At 1,076 μg As(V)/L, plants did not grow, but the growing tips were still alive, whereas plants in 300 μg As(III)/L died.

Only a few reports are available on the effect of phosphate on both As(III) and As(V) toxicity, and the relationship of phosphate concentration to the toxicities is confused because different taxa respond differently. As with *C. parvula*, the toxicity of As(III) to the bacterium *Bacillus cereus* was independent of phosphate concentration, but the toxicity of As(V) could be increased by lowering the phosphate concentration [7]. However, in some fungi, phosphate concentration affects the toxicity of both As(III) and As(V) [8]. In addition, phosphate does not always readily affect As(V) toxicity. Decreases in As(V) uptake in the green alga *Platymonas suecica* occur only when phosphate concentrations reach 100 μM [17].

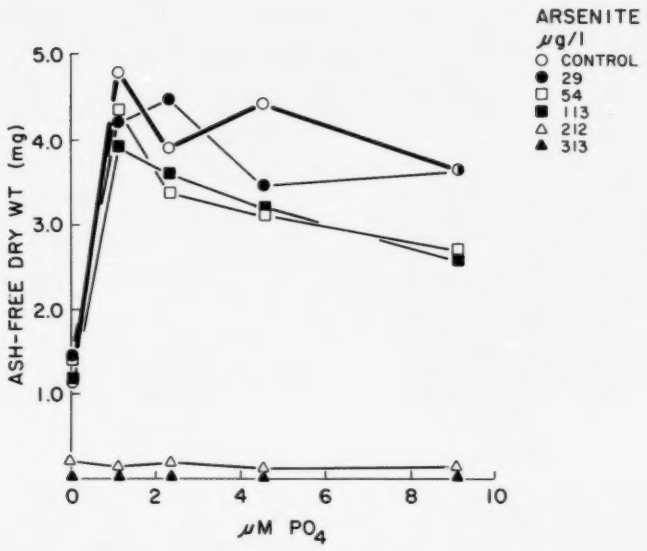

Fig. 2. Growth (n = 5) of *Champia parvula* in response to As(III) (arsenite) at different concentrations of added phosphate. Concentrations of As(III) were measured only once before the plants were added on day 0.

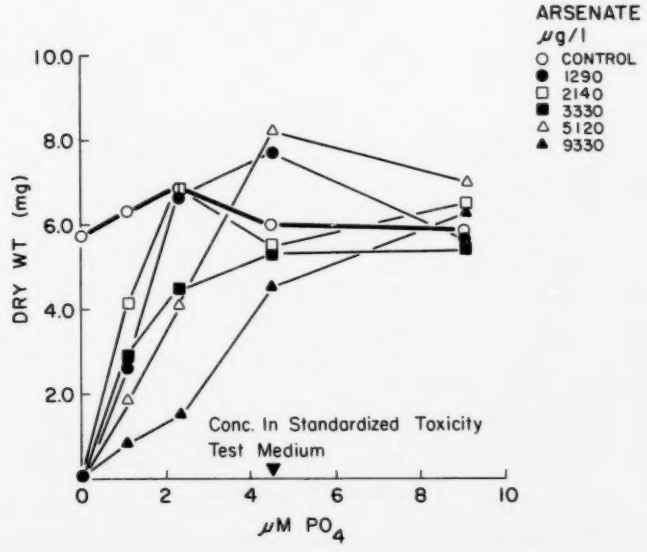

Fig. 3. Growth (n = 5) of *Champia parvula* in response to As(V) (arsenate) at different concentrations of added phosphate. Concentrations of As(V) were measured once prior to adding *C. parvula* on day 0 and again after the media were replaced and fresh As(V) added on day 11.

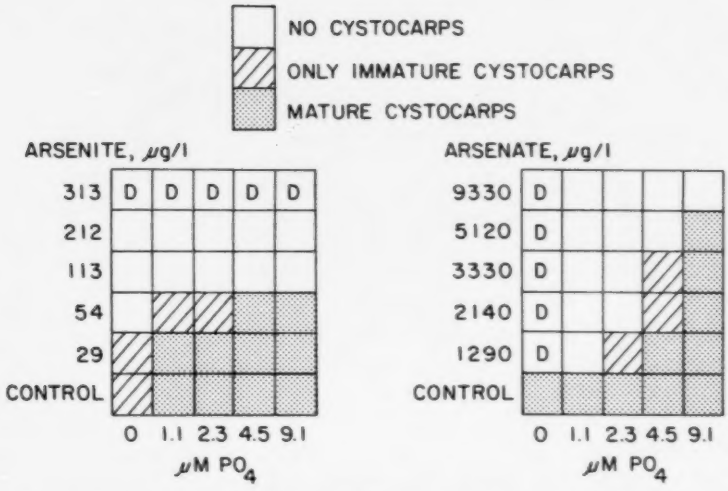

Fig. 4. Sexual reproduction of *Champia parvula* in response to As(V) (arsenate) and As(III) (arsenite) at different concentrations of phosphate. These are the same plants as in Figs. 2 and 3. Cystocarps are evidence of sexual reproduction, and immature cystocarps do not contain spores. The letter D denotes plants that were dead.

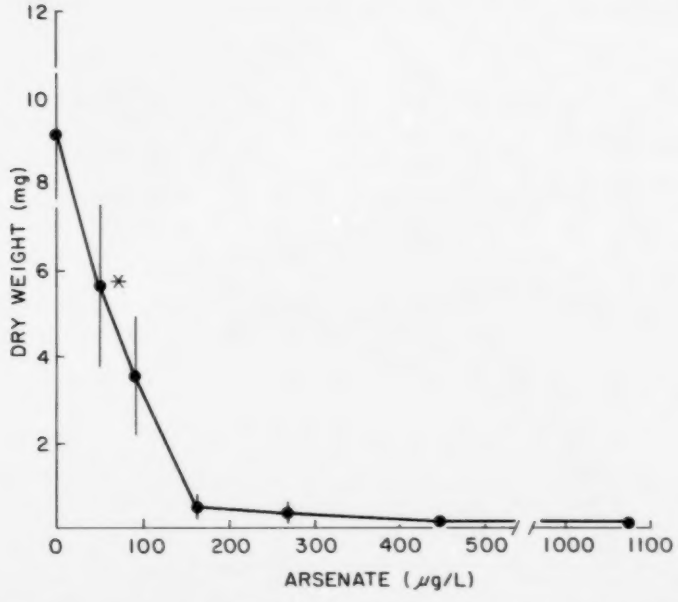

Fig. 5. Growth (n = 10) of *Champia parvula* in response to As(V) (arsenate) when no phosphate was added to the medium. Plants were still alive at the highest concentration tested. Concentrations of As(V) were measured twice, once on day 0 and again on day 7 after the media were replaced and fresh As(V) was added. Asterisk denotes the first treatment in which growth was statistically different from that in the control.

The relationship of laboratory findings with *C. parvula* to predictions of possible effects in the field is complicated by the environmental chemistry of arsenic. Added As(III) is oxidized to As(V) in aerobic seawater and this oxidation is greatly enhanced in the presence of bacteria [18]. Added As(V) can also be reduced to As(III) by bacteria [19], as well as certain corals [20], under aerobic conditions. However, in aerobic aquatic environments, the more prevalent form of inorganic arsenic is believed to be As(V) [21]. Therefore, arsenic in the form of As(III) may not be a serious pollution problem for marine red algae except where it is chronically exposed to it. Because As(V) is more toxic than As(III) when phosphate concentrations are low, this marine red alga would be less sensitive to As(V) under eutrophic conditions.

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